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Editorial

The ever changing landscape of Zika virus infection. Learning on the fly



Zika virus (ZIKV) has gone a long way since its humble discovery in a *Rhesus* sentinel primate in the Zika Forest of Uganda in 1947, to become a potentially devastating international health threat nowadays.¹

For more than half a century ZIKV virus seemed to pose little threat to human beings and for that reason, epidemiological, clinical and therapeutic advances for ZIKV infection have been slow, as a reflection of the relatively small amount of research carried out on the subject as compared with other mosquito-borne viral diseases.

Historically restricted to Africa and Asia, outbreaks with variable impact of autochthonous Zika Fever (ZIKF) were reported in the Pacific region since 2007 and then, as predicted from the distribution of the vectors *Aedes aegypti* and *Aedes albopictus*, ZIKV began to infect patients in South America in 2014, and eventually spread to Central and North America.² The first molecularly confirmed case of Zika virus infection in the Americas Region was reported from Brazil in March 2015.³

In contrast to the relatively slow spread of Ebola virus through West Africa, the ZIKF epidemics in the Pacific and Americas regions moved very rapidly. Indeed, based on the severity of the health threat associated with the continuing spread of ZIKV disease in Latin America and the Caribbean, on 1 February 2016, the World Health Organization declared Zika virus-associated clusters of microcephaly and related neurological disorders a 'Public Health Emergency of International Concern'.⁴

The number of patients affected by ZIKF in the Western Hemisphere increased sharply. As of August 22, 2016 45 countries and Territories throughout the Americas have reported more than 577,000 locally-acquired cases, with estimates of several million real cases, over a period of about one year and a half.⁵ Mathematical models have estimated the basic reproduction number (R_0), or number of cases one case generates on average over the course of its infectious period in an otherwise uninfected population, for ZIKV in the region from 1.4 to 6.6 as key measure of transmissibility in a number of settings.⁶

The underlying reasons for the observed global spread of ZIKV are not fully understood highlighting how little we still understand about the global spread of flaviviruses and other vector-borne diseases. Hypotheses currently under consideration include possibilities as diverse as adaptive evolution of ZIKV allowing for an enhanced infectivity to urban *Aedes* species vectors, or alternatively, adaptive evolution in the human host, leading to higher levels of viremia which would instead enhance both the transmission by the biting mosquitos and the risk of transplacental fetal transmission. Not least important appears to be the

introduction of ZIKV into naive populations of new areas fueled by increased global travel, expansion of tropical urban centers, and abundant susceptible mosquito populations.

The Western hemisphere has faced an unprecedented epidemiological situation with the widespread cocirculation of Dengue (DENV), Chikungunya (CHIKV) and Zika (ZIKV), three arboviruses of major public health importance. The simultaneous emergence and reemergence of this three agents present new challenges to both clinicians and public health authorities.⁷

Overlapping clinical features between diseases caused by ZIKV, DENV and CHIKV, as well as the possible cross-reactivity between DENV and ZIKV and other flaviviruses when immunological methods are used for IgM detection, hamper the reliable diagnosis of the illness in areas with active circulation of both viruses.⁸ Compounding this situation even further is the possibility of coinfections with any combination of them in the human host.^{8,9,13} To the present, experimental dual infection in mosquitos has only been established with Chikungunya and Dengue.¹⁰

With the high possibility of IgM and IgG cross-reactivity in immunoassays, particularly between DENV and ZIKV, differential diagnosis via detection of viral RNA is critical for identifying the causative agent of these infections in The Americas' context.¹¹ The latter is clearly exemplified by the report from Villamil-Gómez et al.¹² in the current issue describing a pregnant Colombian woman with triple coinfection (ZIKV-DENV-CHIKV). A recent publication from several of the coauthors has already shown that coinfection in some endemic areas may be as high, or even higher than monoinfections.¹³ However, the use of diagnostic technologies more affordable to developing countries, such as nested PCR, may pose a higher risk of false positive results as compared to real-time PCR; therefore, some results would require confirmation by other validated techniques like RT-PCR, and/or viral sequencing, as performed in the case published herein.¹² Simultaneous qualitative detection and differentiation of RNA from ZIKV, dengue virus, and chikungunya virus in human sera or cerebrospinal fluid, and qualitative detection of ZIKV virus RNA in urine and amniotic fluid (each collected alongside a patient-matched serum specimen) can be accomplished by the CDC Trioplex rRT-PCR assay recently approved by the USA Food and Drug Administration agency.¹⁴

An interesting particularity of ZIKV is its capacity to be sexually transmitted in addition to the possibility of perinatal and transplacental transmission during childbirth and possibly, by blood transfusion.^{15,16} Evidence of sexual transmission was first suggested in 2011¹⁷ and later confirmed by several reports^{18,19} Although in only few instances the patients involved experienced hematospermia, such as the case reports by Foy and Musso^{17,20}, the

report by Torres JR, et al.²¹ included in this issue, and a parallel one by Huits RMHG, et al.²² confirm and indicate that microhematospermia may be a relatively common event. In fact, of four patients convalescent of recent ZIKV infection followed prospectively to assess the kinetic of the virus in semen, one experienced asymptomatic patent hematospermia, and three others microhematospermia, suggesting that men with acute infection may develop inflammatory changes in the urogenital tract that would play a potential role in the shedding and persistence of the virus in this body compartment with viral loads higher than concurrent sera levels.²² No other arbovirus has shown any association with hematospermia or is found in the semen. Moreover, the demonstration of infectious ZIKV in urine, along with the detection of ZIKV RNA in urine even after viremia has cleared, could be consistent with ZIKV replication in urogenital tissues.^{17,23,24} Of note, at least two instances of sexual transmission from a ZIKV carrier who never showed symptoms has been reported.^{25,26}

Although mosquito bite represents unquestionably the main mode of transmission of Zika, the low viremia observed in many patients and the rapid spread within and among countries in some regions like the Americas supports a role for other modes of transmission, such as sexual intercourse, that could contribute to its rapid spread.²⁷ As identifying and characterizing cases of sexually transmitted Zika virus infection in areas experiencing intense autochthonous vector-borne Zika virus transmission is a difficult task, most reports of sexual transmission originate in areas where autochthonous transmission is not occurring which allows for unique and important opportunities to learn about this emerging mode of transmission.

The immune-privileged nature of the testes may allow ZIKV to persist in this tissue for prolonged periods, serving as reservoirs with the potential to initiate new transmission cycles from otherwise healthy individuals. Seminal shedding of ZIKV seems to coincide with the duration of spermatogenesis (69–80 days), suggesting the possibility of infection of sperm progenitors and viral shedding during the differentiation process.²² Of note, immunodeficient mice with experimental ZIKV infection show the highest levels of viral replication in the testes and brain.²⁸ However, further studies aimed to understand persistence of ZIKV in semen and the real risk of ZIKV sexual transmission are necessary.

The European Center for Diseases Control currently recommends deferral of semen donation for 28 days after returning from areas where ZIKV is endemic.²⁹ Of note, in one recently published case ZIKV RNA was detected in semen up to 134 days indicating a prolonged potential risk for sexual transmission, for a period longer than previously reported.³⁰ These latest findings may suggest a revision of the recommendations of prevention of sexual transmission and infection in pregnancy issued by current health authorities. Indeed, very recently WHO officially advised that all men and women returning from areas where transmission of Zika virus is known to occur should adopt safer sex practices or consider abstinence for at least 6 months upon return to prevent Zika virus infection through sexual transmission.³¹ By comparison, suspected sexual transmission of Ebola virus has occurred 179 days after onset of the disease and Ebola virus RNA has been detected in semen for 4–6 months after disease onset in more than 40% of survivors.^{32,33}

Little information is available on the cellular tropism of ZIKV and the nature of the cellular receptors that mediate its entry in different tissues, as well as the mechanisms of ZIKV infection and the signaling pathways and antiviral immune response of the host elicited by this virus. In this respect, the work by Chen J, et al. revealing that uterine fibroblasts (UF) are susceptible to infection opens the possibility of a relevant role for the uterus as a conduit for virus during heterosexual intercourse.³⁴

These results are in accordance with earlier and recent reports in the literature underscoring the importance of fibroblasts as a primary cell type of replication for flaviviruses, in close association with host cell membranes, that may contribute to subsequent viral dissemination.^{35,36} The primary infection of skin fibroblasts with ZIKV is linked to the up-regulation with TLR3 mRNA expression and increased transcription of RIG-I and MDA5, all of which are related to innate immune responses to RNA virus infections. This is followed by an increase in the expression of interferon-alpha and -beta, and their downstream pathways of immune activation.³⁶ Additional investigations are needed to define the exact contribution of specific fibroblasts cell receptors and/or attachment factors to ZIKV infection, tropism and pathogenesis.

Another paper included in this issue by Coelho F, et al.³⁷ from Rio de Janeiro, Brazil, indicates that, in some contexts, women of reproductive age groups are far more likely to get Zika than men; suggesting the possibility that men-to-women sexual transmission may account for this difference. A caveat to this conclusion is the fact that just because the statistical data shows an apparent correlation between these two parameters it doesn't mean that there is necessarily an underlying causal relationship; besides, female to male sexually transmitted Zika virus has been also described recently. Indeed, the confirmation of at least one case of ZIKV infection transmitted from a symptomatically infected woman to a male sex partner,³⁸ and the detection of ZIKV RNA in vaginal fluids of a patient 3 days after symptom onset and in cervical mucus up to 11 days,³⁹ indicates that partners of infected women, might acquire ZIKV through exposure to vaginal secretions or menstrual blood during sexual intercourse. Therefore, other factors would explain in part why women in the sexually active age bracket may be more affected.

As illustrated by the paper of Machado-Alba JE, et al. also found in this issue,⁴⁰ Guillain Barre Syndrome (GBS) appears to be a genuine risk in patients recently infected with ZIKV. This association was first reported during the outbreak of ZIKV in French Polynesia between 2013 and 2014 (risk: 24 per 100,000 ZIKV infections).⁴¹ The risk of GBS reported was 10 times the annual risk in the USA (1.8 per 100,000). Most cases occurred in symptomatic patients with a median of 6 days of symptoms in 88% of the patients.⁴¹

According to PAHO, an apparent increased incidence of GBS has also been observed in Brazil, Colombia, and El Salvador in association with the current epidemic of ZIKV in the Americas.⁵ To date, such a rise in the incidence of GBS cases has been reported in at least 12 countries and territories with laboratory confirmed cases of ZIKV.⁵ Mortality of ZIKV and GBS is expected to be higher in medical environments where respiratory support is not available or scarce, as is the case in many locations of Latin America. It should be noted that changes in the incidence of GBS in the region may not be related exclusively to ZIKV, but also to fluctuations in the incidence of DENV and CHIK, or other viral and bacterial pathogens.

Finally, the work by Rodriguez-Morales A, et al.⁴² published herein represents one of the first to use of Geographic Information Systems (GIS)-based epidemiological maps in a Zika epidemic. The incorporation of these technologies would allow to integrate preventive and control strategies, as well as public health policies, for joint control of this and similar vector-borne diseases in any area of a country. As dengue, CHIKV and ZIKV widely cocirculate in the continent nowadays; updated GIS maps of these infections as well as coinfections will be potentially useful.

GIS-based technologies drawing on local census data may generate useful information showing public health experts where to target prevention efforts, particularly among women of child-bearing age and their partners. By allowing to monitor the spread of the ZIKV, they can properly dole out domestic assistance and

resources to the areas with the greatest number of cases. Such maps may also provide relevant information aimed to assess the risk of travelers to specific destinations in highly transmission areas allowing detailed prevention advices.

As health specialists need reliable information to make sound decisions about what preventive actions to take, the use of GIS collected information to locate the most vulnerable populations in a given region represents an important stride than can help in educating people on the risks of the ZIKV and bring actions that can protect health and curb the spread of disease.

Undoubtedly, protection of exposure of susceptible individuals to ZIKV and suitable vector control actions are the only tools available to reduce the advance and impact of this infection at the present moment, especially until effective vaccines and antiviral therapy become available.

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